Orthotopic Liver Transplantation for Acute Grade IV Hepatic Graft-Versus-Host Disease Following Bone Marrow Transplantation

To the Editor: Severe graft-versus-host disease (GVHD) occurs in nearly 30% of HLA-identical bone marrow transplant (BMT) recipients and increases to 60–80% when a partially matched family donor or an unrelated marrow is used. After the skin, the liver is the most frequently involved target of acute GVHD. Refractory liver GVHD disease progresses to a profound cholestasis with destruction of small bile ducts due to an immunological assault on bile duct epithelium. Once all bile ducts disappear, the lesion is considered irreversible and leads to hepatic failure. Replacement of the liver may theoretically be the only therapeutic option, but patients must be carefully selected using the following criteria: 1) good performance status; 2) low risk of primary disease relapse; 3) irreversible liver disease; and 4) absence of other organ dysfunction, mainly infection or GVHD.

A 31-year-old woman diagnosed with myelodysplastyc syndrome underwent an allogeneic BMT from an HLA-identical sibling donor. The preparative regimen consisted of cyclophosphamide (120 mg/kg) and fractioned total body irradiation (12 Gy). The GVHD prophylaxis employed was short methotrexate and cyclosporine A (CsA). A successful engraftment occurred on day +22 and an erythematous rash on her palms and soles with mild diarrhea and increasing jaundice began. With an overall clinical grade III acute GVHD, she was treated with methyl-prednisolone (MP): 2 mg/kg on day +25. Skin and gut GVHD improved, but the liver showed progressive cholestasis (Table I). On day +41 after BMT, antithymocyte globulin (ATG) was added at 15 mg/kg every other day for six doses, without response (Table I). A transjugular liver biopsy on day +50 showed complete absence of bile ducts and no evidence of lymphocyte infiltrates. After a month's waiting time she underwent a liver transplant on day +109 after BMT. Preoperatively the donor received 1 g of intravenous MP and during removal the liver was perfused with OKT3 (5 mg). To prevent rejection or GVHD after transplant CsA was continued and the MP dose was increased to 2 mg/kg/day for 2 weeks with subsequent tapering. Chimerism study by Southern blot (YNH24, TBQ7, and CMM101 probes) and polymerase chain reaction (PCR) (YNZ22, 33.6′6 locus) of variable number tandem repeats of DNA isolated from peripheral blood leukocytes on day +161 after BMT and +52 after liver transplant showed only bone marrow donor origin. The patient was discharged home on day 26 after liver transplant and by day +36 she had a Karnofsky score of 90%, marrow graft function was preserved, and liver function tests were normalized (Table I). A week later she began with new clinical signs of gut GVHD and prednisone dose was again increased with improvement. On day +82 after liver transplant the patient suffered a disseminated cutaneous varicella that was treated with high-dose acyclovir. Fulminant septic shock with necrotic cutaneous lesions occurred on day +129 after liver transplant and +238 after BMT and the patient died. Autopsy showed disseminated aspergillosis with preferential involvement of skin and lung. The gastrointestinal tract showed residual nonspecific lesions and the liver had diffuse mild sinusoidal dilatation without inflammation or necrosis.

Only a few post-BMT liver transplants have been reported for either severe veno-occlusive disease [1–4] or GVHD [5,6]. Published experience suggests that liver rejection is not the main problem and could be prevented by maintaining post-BMT immunosuppression, although some additional measures must be taken to avoid allogeneic reactions coming from the new graft. Infection and problems related to pancytopenia affected outcome adversely in some reported cases [2–5]. In our case underlying gut GVHD activity was probably underestimated before liver transplant, and its reactivation called for more intense and prolonged immunosuppression; serious infection occurred, and finally death. In conclusion, a liver transplant may be an option in end-stage liver GVHD, but patients must be very carefully selected. This case suggests that silent but active GVHD in the gut should be carefully excluded and considered another contraindication.

Acknowledgments

This work was supported in part by grant FIS 93/0522 from the Fondo de Investigaciones Sanitarias de la Seguridad Social, National Institute of Health of Spain.

Angela Figuera
José F. Tomás
BMT Unit
Mariá J. Otero
Hematology Department, Hospital Universitario La Princesa
Enrique Moreno
Ismael García
Liver Transplant Unit, Surgery Department, Hospital 12 de Octubre
José M. Fernández-Rañada
Hematology Department, Universidad Autónoma, Madrid, Spain

References

1. Nimer SD, Milewicz AL, Champlin RE, Busuttil RW: Successful treatment of hepatic veno-occlusive disease in a bone marrow transplant patient with orthotopic liver transplantation. Transplantation 49:819–821, 1990.
2. Rapoport AP, Doyle HR, Starzl T, Rowe JM, Doeblin JM, DiPersio JF: Orthotopic liver transplantation for life-threatening veno-occlusive disease of the liver after allogeneic bone marrow transplant. Bone Marrow Transplant 8:421–424, 1991.
3. Tydén O, Wennberg L, Soderdahl G, Erlezon BO, Ringdén O: Liver transplantation for hepatic veno-occlusive disease after BMT. Abstracts of the XIX Annual Meeting of the European Bone Marrow Transplant Group, Garmish-Paterkirschen. Bone Marrow Transplant 12(suppl 2):77 (abstract), 1993.
4. Tischler HJ, Ringel B, Dietrich H, Beltoni C, Freund M, Schhlitt HJ, Tietz S,

TABLE I. Patient Data*

	Day after BMT/OLT							
	+20	+33	+40	+85	+110/0	+114/5	+133/24	+238/129
Bil T/C (mg/dl)	3/1.8	26/15	40/23	52/25	45/24	22/12	2.8/1.3	1.5/0.7
AST (units/liter)	126	93	138	119	134	16	13	13
GGT (units/liter)	297	920	1,100	1,380	1,400	126	101	289
Alkaline phosphatase (units/liter)	619	1,146	1,555	2,000	3,400	109	272	166
Prothrombin activity (%)	92	93	85	94	86	75	100	99
Albumin (g/dl)	3.3	2.6	2.8	2.9	2.7	4.3	4.3	3.3
WBC/mm^3	900	3,600	4,200	4,400	2,200	3,830	9,000	1,000
Platelets/mm^3	34,000	137,000	119,000	31,000	19,000	21,000	17,000	3,300

*BMT, bone marrow transplantation; OLT, orthotopic liver transplantation; Bil T/C, bilirubin total and conjugated; AST, aspartate aminotransferase; GGT, gammaglutamyltranspeptidase; WBC, white blood cell count.

Kuse E, Maschek H, Franke A, Link H: Successful orthotopic liver transplantation for a patient with veno-occlusive disease of the liver after BMT. Abstracts of the XIX Annual Meeting of the European Bone Marrow Transplant Group, Garmisch-Paterkirschen. Bone Marrow Transplant 12(suppl 2):77 (abstract), 1993.
5. Rhodes DF, Lee WM, Wingard JR, et al: Orthotopic transplantation for graft versus host disease following bone marrow transplantation. Gastroenterology 99:536–538, 1990.
6. Marks DI, Dousset B, Robson A, et al: Orthotopic liver transplantation for hepatic GVHD following allogeneic BMT for chronic myeloid leukemia. Bone Marrow Transplant 10:463–466, 1992.

Spurious Automated White Cell Count With Coulter STKS in the Myelodysplastic Syndromes Suggests the Presence of a Red Cell Membrane Defect

To the Editor: Accurate automated white cell counting requires complete red cell lysis in the white cell channel(s). Commercial lysing solutions exploit different methods of lysis. For example, the Coulter STKS utilizes a hypotonic solution and the Technicon H-1 uses a detergent, while the Cell-Dyn 3500 uses both methods, each in a separate channel [1]. Hypotonic lysis of red cells with increased osmotic resistance, e.g., neonatal red cells, may be incomplete [1,2]. We, among others [2], have encountered difficulty with automated white cell counting of neonatal blood using the Coulter STKS due to incomplete lysis of red cells. In addition to neonatal blood, problems with incomplete lysis of red cells have been seen in post-splenectomy states, megaloblastic anemia, hemoglobinopathies, and liver disease [1,3]. We would like to extend this list to include the myelodysplastic syndromes (MDS).

We have experienced difficulty with the absolute and/or differential automated white cell count in two patients with MDS.

Case 1

The first case was a 66-year-old man with refractory anemia and the following complete blood count parameters: red blood cells 2.13 million/μl, hemoglobin 7.6 g/dl, hematocrit 22.1%, mean corpuscular volume 103.9 fl, mean corpuscular hemoglobin (MCH) 35.6 pg, MCH concentration 34.2%, red cell distribution with index (RDW) 18.2, and platelet count $7 \times 10^3/\mu l$. Using the Coulter STKS, the white blood cell (WBC) count was $4.6 \times 10^3/\mu l$ with an automated differential of 30.4% neutrophils, 65.7% lymphocytes, 3.5% monocytes, 0.1% eosinophils, and 0.3% basophils. The differential count was flagged by the instrument, and the scattergram showed the presence of prominent debris in the low-volume portion of the lymphocyte area. The specimen was repeated on the Technicon H-1, which gave a total WBC count of $5.1 \times 10^3/\mu l$ and a differential count of 60.9% neutrophils, 30% lymphocytes, 4% monocytes, 0.1% eosinophils, 0.2% basophils, and 4.9% large unstained cells (LUC). A manual differential count closely matched that of the Technicon H-1.

Case 2

The second case was an 80-year-old woman with refractory anemia with excess blasts and the following complete blood count parameters: red blood cells 3.01 million/μl, hemoglobin 10.9 g/dl, hematocrit 31.8%, mean corpuscular volume 105.4 fl, MCH 36.4 pg, MCH concentration 34.4%, RDW 15, and platelet count $91 \times 10^3/\mu l$. With the Coulter STKS, the WBC count was $1.7 \times 10^3/\mu l$ with an automated differential of 17.8% neutrophils, 77.4% lymphocytes, 1.4% monocytes, 3% eosinophils, and 0.4% basophils. The instrument had no qualitative flags. However, the scattergram again showed contamination of the lymphocyte area by debris of much lower volume. The Technicon H-1 gave a total WBC count of $2 \times 10^3/\mu l$ with a differential count of 30.9% neutrophils, 56.9% lymphocytes, 1.6% monocytes, 6% eosinophils, 0.5% basophils, and 4.1% LUC. A manual differential count was essential similar to the Technicon H-1 count. Hemoglobin F measurement in both cases was $< 1\%$. In both cases the error associated with incomplete red cell lysis led to a spurious increase in the lymphocyte count and a decrease in the neutrophil count.

Osmotic fragility of red cells is related to the ratio of mean surface area (MSA) to mean cell volume [3]. Neonatal and post-splenectomy red cells have a higher content of membrane phospholipids and cholesterol, leading to an increased MSA and increased red cell resistance of hypotonic lysis [3]. Red cell membrane abnormalities are virtually unstudied in MDS. A small number of studies suggest abnormalities in membrane proteins such as spectrin, band 4.1 protein, and glycophorin C-band 4.1 protein interaction, leading to changes in osmotic fragility [4,5]. We suggest that abnormal automated white cell differential counts due to incomplete lysis of red cells by hypotonic reagents in elderly patients with unexplained cytopenias should raise the possibility of an underlying MDS. In addition, this phenomenon may help identify an interesting subgroup of MDS patients with possible abnormal red cell membrane for further studies.

M. Tarek Elghetany
S. David Hudnall
Division of Hematopathology, Department of Pathology, The University of Texas Medical Branch, Galveston, Texas

References

1. Dorner K, Schultz S, Reinhardt M, Seeger H, van Hove L: Improved automated leucocyte counting and differential in newborns achieved by the hematology analyser Cell-Dyn 3500®. Clin Lab Haematol 17:23, 1995.
2. Fournier M, Adenis C, Fontaine H, Carnaille B, Goudemand J: Evaluation of the white blood cell differential provided by the Coulter STKS® in a children's hospital. Clin Lab Haematol 16:33, 1994.
3. de Haan LD, Werre JM, Th Ruben AM, Huls HA, de Gier J, Staal GEJ: Alterations in size, shape and osmotic behaviour of red cells after splenectomy: A study of their age dependence. Br J Haematol 69:71, 1988.
4. Ideguchi H, Yamada Y, Kondo S, Tamura K, Makino S: Abnormal erythrocytes band 4.1 protein in myelodysplastic syndrome with elliptocytosis. Br J Haematol 85:387, 1993.
5. Maeda N, Nakajima T, Izumida Y, Suzuki Y, Tateishi N, Seiyama A: Decreased deformability of red cells in refractory anemia and the abnormality of the membrane skeleton. Biorheology 31:395, 1994.

Cerebral and Vein Thrombosis, Transient Protein S Deficiency, and Anticardiolipin Antibodies

To the Editor: We report the case of a young woman with cerebral venous thrombosis associated with the presence of anticardiolipin antibodies and a transient protein S deficiency, suggesting a role for the antiphospholipid antibodies (aPL) in reducing the free protein S levels, thus determining hypercoagulable state with cerebral and venous thrombosis.

aPL have been found to be associated with recurrent spontaneous abortions and systemic arterial and venous thrombosis, as well as thrombosis of the cerebral vessels [1,2]. Recently, cases of deep vein thrombosis or severe diffuse thromboembolic disease associated with the transient presence of anticardiolipin antibodies (aCL) and functional protein S deficiency have been reported [3], suggesting a new, possibly autoimmune mechanism [4] for the thrombosis in the primary aPL syndrome. Low levels of free